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14. ABSTRACT

The major goal of this project is to determine and compare the overall dynamic architectures of both ERs bound to different known DNA response elements (EREs). This study combines small-angle X-ray solution scattering (SAXS) data and computational modeling to address the multimeric assembly of ER DBD-hinge-LBD complexes. To date, we have successfully modeled ERβ CDE bound to its cognate DNA binding site and generated homogenous ER DBD-hinge-LDB protein samples (ERβ CDEF) needed for SAXS studies. We have also collected SAXS data on ERβ CDEF, but not ERβ CDE. We will continue to generate and compare the ERβ□CDE and CDEF fragments for small angle x-ray scattering (SAXS) analyses of ER multidomain fragments ± EREs and various peptides to obtain solution structure information. Having worked out the conditions for the expression and purification of ERβ CDEF, we do not expect difficulties in the expression and purification of ERβ CDE domain. By integrating SAXS analyses and computational biology, we expect to better understand ER multi-domain assembly mechanisms. It is anticipated that this information will help explain how domain interfaces in ER/ERE complexes modulate ligand-dependent transcriptional activity in response to various SERMs, providing novel structural insights that will facilitate improved targeting of ER-positive breast cancers, especially those that are refractory to current adjuvant therapies.

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Introduction

This study combines complementary computational modeling and SAXS expertise (S.Y.) with expertise on SERM-mediated ER LBD structure and signaling (G.G.). It is anticipated that our studies on the DBD-hinge-LDB assembly in ERα and ERβ will determine the extent to which inter-domain interfaces control ligand-dependent transcriptional activity in response to various known agonists, antagonists, or novel SERMs, providing structural insights that can be exploited for novel targeting of ER-positive breast cancers.

For clarity and completeness, I will first list the specific aims of this DoD grant, followed by a summary of the studies and results within the first 12 months from the CWRU subcontract award as well as future plans for the next 12 months.

Body

A. Specific Aims

Aim 1. Determine structural models of assembly states of the DBD-hinge-LBD complexes in ERα and ERβ using computational modeling.

1a. Develop an all-residue coarse-grained (ARCG) model for molecular dynamics simulations. Test the ARCG model on known ER LBD homodimers[1] first and then apply it to ERα and ERβ DBD-hinge-LBD complexes.

1b. Design a hierarchical structural clustering algorithm and apply it to structural modes generated from ARCG simulations in Aim 1a. Determine a small number (~10) of assembly states using a two-step procedure that takes into account both differences in detailed residue-residue distances and overall macromolecular scattering.

1c. Compute theoretical SAXS profiles for the resulting assembly states obtained in Aim 1b by using a high-throughput, scattering computing program of “Fast-SAXS”. Compare scattering differences and examine theoretical DBD-LBD interaction mechanisms of assembly states.

Aim 2. Detect ER shape changes in response to small molecules using SAXS experiments.

2a. Express ERα and ERβ DBD-hinge-LBD plasmid constructs in bacteria and purify them to a concentration of approximately 1 mg/ml. Determine the sample size distribution using dynamic light scattering. Synthesize and prepare various ligands and different ERE oligomers as agents to elicit ER conformational changes.

2b. Collect SAXS data for ERs at the APS and NSLS. SAXS experiments will be conducted with ER fragments in the presence of several ligands, including tamoxifen, 17β-estradiol (E2), raloxifene, and various known ERE oligomers. Examine differences in experimental SAXS patterns and map out the global shape changes upon the binding of ligands with diverse behaviors.

Aim 3. Determine DBD-LBD interactions and quantify molecular mechanisms of ER multi-domain assembly.

3a. Use BSS-SAXS as a structural technique[2-3] to determine ER multi-domain assemblies. Combine structural models of assembly states obtained from computational modeling in Aim 1 and experimental SAXS data obtained in Aim 2 to quantify the population state of each multi-domain assembly state using a Monte Carlo procedure. Determine DBD and LBD interaction mechanisms under various conditions.

3b. Use hydrogen/deuterium (H/D) exchange analysis to define specific local molecular details of DBD-LDB interfaces and identify structural features of hydrophobic forces and hydrogen-bonding at DBD-LDB interfaces. Resolve the multimeric structural assembly mechanisms in ERα and ERβ in response to various signals. Make specific theoretical predictions of sites at the interface(s) that stabilize specific assembly states for functional studies. Use site-directed mutagenesis to confirm predictions. Make predictions of sites at the interface(s) that can be cross-linked to stabilize specific assembly states for future crystal studies to capture such structural snapshots of energetically favorable assembly states.

B. Studies and Results

The results described here include progress since this sub-award was made in April 2011. We have make progress with the simulations of ERβ DBD-hinge-LDB (also known as CDE) in **Aim 1a**, structural clustering analyses of simulation trajectories in **Aim 1b**, and SAXS computing in **Aim 1c.** In addition, we have collected SAXS of ERβ CDEF in complex with ERE oligomers and ligands, as proposed in **Aim 2**.

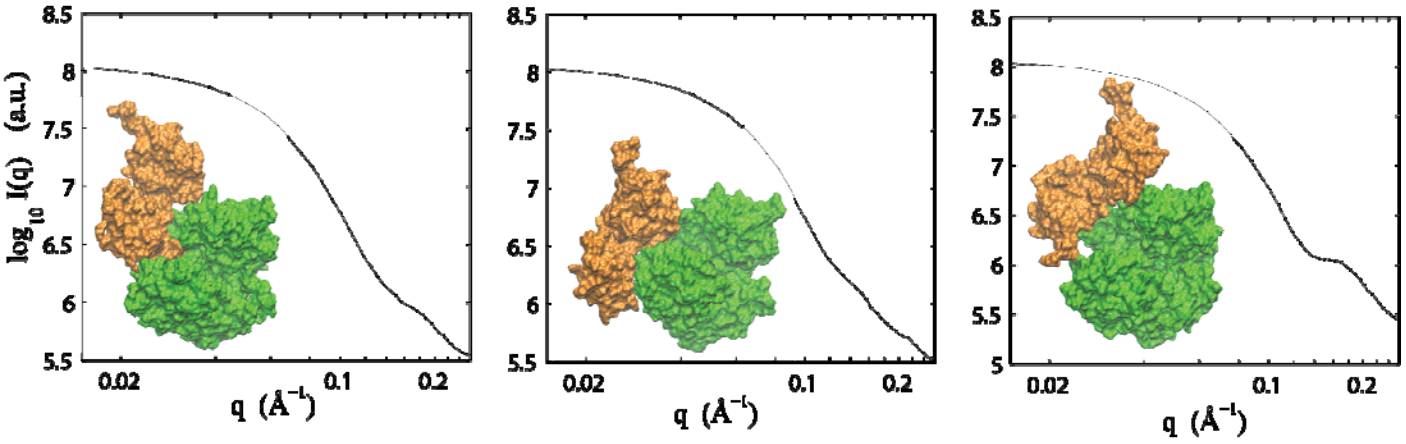

Figure 1. Three stable conformations have been identified from m olecular dynamics simulations of ERbeta CDE. Their theoretical SAXS curves are also computed using in-house Fast-SAXS software (Ref. 4).

To demonstrate the results from molecular dynamics (MD) simulations (**Aim 1)**, **Figure 1** shows three energetically stable conformations observed for the ERβ DBD-Hinge-LBD (CDE) fragment. This is achieved by 100 separated MD simulation trajectories, and each has 10 microseconds, resulting in a total of 1 millisecond. All the resulting MD configurations are clustered into a small number of conformational clusters using a two-step procedure. For the SAXS computing, our in-house Fast-SAXS package was used for all the three conformations. The results in **Figure 1** show that each SAXS profile I(q) is different from the others, suggesting experimental SAXS data can be a very powerful tool for

determining their population fractions. A previously published SAXS-based shape reconstruction method will be used once the SAXS data of this CDE complex (Ref. 2).

For **Aim 2**, the expression and purification the CDE and CDEF domains of ERβ have now been largely optimized and we are able to consistently purify up to 10 mg of protein at a concentration of 2 to 3 mg/ml (**Figure 2**). Higher concentrations have been more difficult to obtain due to protein precipitation. However, these concentrations are sufficient for SAXS analyses. We have also found conditions under which the purification can be performed without ligand. Therefore the same protein preparation can be used for SAXS measurement with or without the addition or different ligand such as estradiol and 4-hydroxytamoxifen (OHT) as well as ERE oligomers of different lengths and sequence, thus allowing direct comparison between the same SAXS samples. Initial experiments were carried out with the CDEF fragment of ERβ, which contains an additional 25 amino acids that are thought to be at least partially unstructured. It will be important to compare these two ERβ fragments by SAXS because no existing structural data exists for the F domain of either ER subtype.

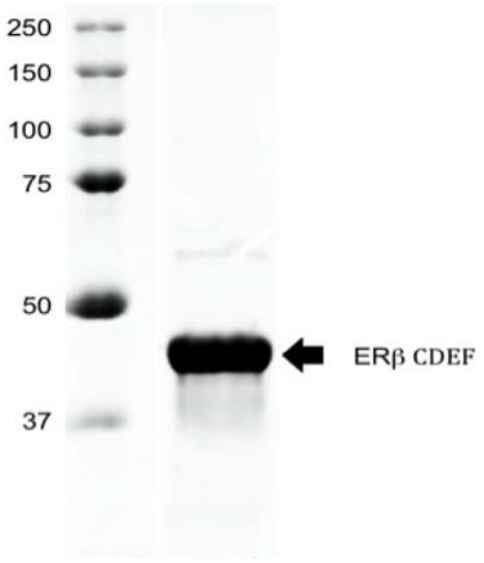

Figure 2. ERβ CDEF protein is visualized by Coomassie blue staining on an SDS-PAGE gel after purification

For **Aim 2b**, purified ERβ CDEF fragment has been analyzed by SAXS in the presence and absence of a known oligomeric ER DNA binding site (ERE). Figure 3 shows the SAXS data for this polypeptide in the presence of ERE, demonstrating that we have been able to obtain high-quality SAXS data for this functional complex. Shown is the plot of I(q) vs q (in a logarithmic scale), where q is the amplitude of X-ray momentum transfer during the scattering. A triplicate of 20 uL of sample was continuously flowed through a 1 mm diameter capillary and each was exposed for 30 sec. The data were recorded under the condition of the X-ray energy of 14 keV and the temperature of 10 degree. This result lays the foundation for further studies of the receptor in the presence of different ligands and different specific DNA sequences that are associated with naturally occurring ER target genes.

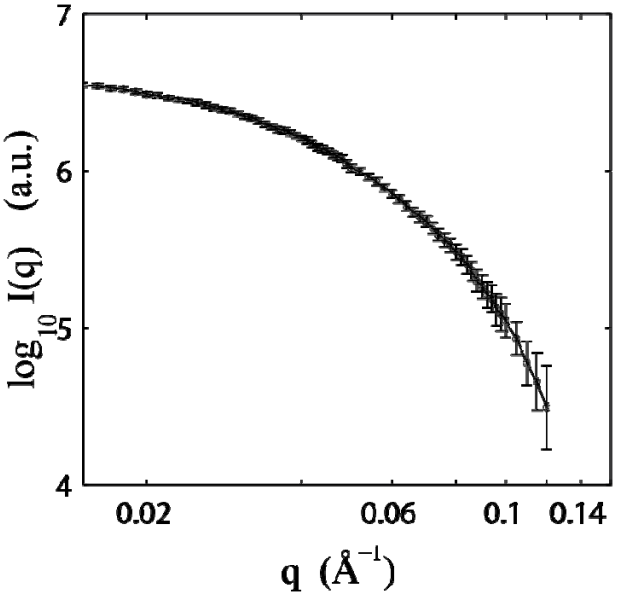

Figure 3. Experimental SAXS of ERbeta CDEF in the presence of DNA. Data were collected at the NSLS in Oct. 2011.

Key Research Accomplishments

- Successful modeling of ERβ CDE bound to DNA
- Successful purification of ERβ CDEF
- Successful collection of SAXS data of apo ERβ CDEF in the presence of DNA at the NSLS

Reportable Outcomes

None

Conclusion

An important goal of this study is to generate homogenous ER DBD-hinge-LDB (also known as CDE) protein samples needed for SAXS studies. Up until now, we have collected SAXS data of CDEF samples, but not that of CDE. We will continue to generate and compare the ER□□CDE and CDEF fragments for small angle x-ray scattering (SAXS) analyses of ER multidomain fragments ± EREs and various peptides to obtain solution structure information. Having worked out the conditions for the expression and purification of ERβ CDEF, we do not expect difficulties in the expression and purification of ERβ CDE domain. Future work will also be focused on optimizing the purification of ERα CDE. ERα protein purification has been more challenging because of the longer hinge region that separate the DBD and LBD that is more prone to proteases digestion.

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Appendices

None

Supporting Data

Included in Body